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UNITED STATES DEPARTMENT OF AGRICULTURE

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(Problem 1)

FINAL REPORT

IDENTIFICATION OF BACTERIAL FLORA IN THE DIGESTIVE TRACT OF THE SOUTHERN PINE BEETLE, DENDROCTONUS FRONTALIS (ZIMM.)

(Cooperative Agreement #19-149 with LSU at Alexandria)

by

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Final Report Summary

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IDENTIFICATION OF BACTERIAL FLORA IN THE DIGESTIVE TRACT OF THE SOUTHERN PINE BEETLE, DENDROCTONUS FRONTALIS (ZIMM.)

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Over 250 isolates of bacteria were recovered from fore-, mid-, and hindgut sections of female and male southern pine beetles collected as they constructed egg galleries in loblolly pine. The isolates were processed through 21-25 standard cultural or biochemical tests to determine identification of species. Because of the large number of possible character combinations and a corresponding large number of possible species, all data for each isolate was entered on computer punch cards and compared to species character codes.

Eleven genera and 23 species were identified and an additional 26 strains were recognized among 6 of the above genera. Many of the strains may be varients of known species but additional tests and numerical analyses are required for proper placement.

The most widely represented genera were Achromobacter, Alcaligenes, Brevibacterium, and Pseudomonas with 5 or more species and strains each. The following genera were represented by 3 or fewer organisms: Bacillus, Sarcina, Enterobacter, Flavobacterium, Kurtnia, Mycobacterium, and Staphylococcus.

It is too early to determine what role these bacteria play in the ecology of the complex association of microbes in the gallery or in the development of the insects. A future study, "Identification of bacterial flora in galleries of the southern pine beetle," FS-S0-2203-1.29, will shed light on those bacteria which may influence the host phloem substrate before and during larval feeding.

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NOTES ON METHODS

The bacterial isolations were made from fore-, mid-, and hindgut sections as stated in the study plan. The number of sections had to be limited because of an unexpected abundance of isolates obtained in the early part of the study and because of the extended time required for careful dissection of each gut section. Thus 20 female and 16 male gut sections were plated from June 4-8, 1973 but only 5 female and 6 male sections were sampled from July 23-27. Over 250 isolates were obtained from the gut sections and processed through 21-25 cultural or biochemical tests to determine species identification. There was concern that part of the large number of isolates represented uncontrolled contamination but a check of the surface sterilization of beetles showed no contaminents.

When the magnitude of the results became apparent assistance of the Biometrics Branch was obtained to computerize the data and group the isolates according to genera and species codes. Because of this assistance, identifications were more objective and strains within genera could also be separated into designated categories (i.e. A, B, C, etc.) when 2 or more characters differed.

RESULTS

A list of all species identified in the study is given in Appendix I and their distribution among the gut sections is given in Appendices II and III. An examination of this data reveals the complexity and abundance of bacterial flora present in the gut of the southern pine beetle engaged in gallery construction. Eleven genera with diverse characteristics are represented by 23 species and 26 recognizable strains. Many of these strains may be varients of known species but it was impossible to match them with standard methods of bacterial classification. Additional tests and possible future numerical analysis may provide a taxon for placement.

The numerical number of different species and strains found in the fore-, mid-, and hindguts of each sex are given below.

	Female			Male		
	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Species	8	8	10	8	10	11
Strains	9	7	6	5	11	10
Total	17	15	16	13	21	21

The totals show that an apparently greater variety of organisms was present in the male mid- and hindgut, but the least variety occurred in the foregut.

GENERA

The distribution among the gut locations and the percent of gut sections containing each genera is given in table 1. This summation shows that Achromobacter occurred more often in the male gut sections and was very common in the male hindgut. Alcaligenes on the other hand was more common in the female mid- and hindgut. Brevibacterium was common on the male mid- and hindgut but absent in the foregut. On the other hand, among the females it was more prevalent in the foregut. The genus Pseudomonas occurred in all gut sections of both sexes but was most common in the male midgut. Bacillus was located in all gut locations but the percentage of occurrence was relatively low. Flavobacterium occurred in all three gut locations of the female but was absent in the male. This may signify female-specificity, but a relatively small number of gut sections harbored the genus. The other genera were found scattered among various locations but again they were in a relatively small number of respective gut sections.

Table 1.--Percent of gut sections containing each genera of bacteria identified from the southern pine beetle

Genera	Female			Male		
	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
	-percent-			-percent-		
Achromobacter	40	38	43	50	67	86
Alcaligenes	30	50	71	33	33	43
Brevibacterium	40	25	14	0	56	71
Pseudomonas	30	25	14	17	56	43
Bacillus	30	25	14	33	22	14
Flavobacterium	10	25	14	0	0	0
Sarcina	0	25	14	33	0	0
Enterobacter	0	0	14	17	0	14
Kurthia	0	0	0	0	0	14
Mycobacterium	0	0	0	17	0	0
Staphylococcus	0	13	0	0	0	0

SPECIES

Although all gut sections sampled in the study contained bacteria only Alcaligenes faecalis and Achromobacter strain "D" were found in the fore-, mid-, and hindgut locations of both female and male beetles (table 2). Five species or strains were found in all three locations of one sex but only 2 locations in the opposite sex. sex. Thus Brevibacterium acetylicum and Pseudomonas strain "B" were present in all locations but the male foregut, Achromobacter erydice and Bacillus cereus in all but the female hindgut, and Achromobacter xerosis in all but the female midgut.

Although none of the bacteria were present in more than 50% of the respective gut samples, a relatively high incidence of some compared to others may indicate a generally permanent association with the southern pine beetle. For example A. xerosis occurred in 44% of the male midguts, B. acetylicum in 43% of the male hindguts, and A. faecalis in 43% of the female hindguts.

Most other species were scattered among gut locations of both females and males, but all occurred in a relatively low percent of the respective locations. One exception was recorded with Brevibacterium lipolyticum present in 44% and 43% of the male midguts and hindguts respectively.

Table 2.--Percent of gut sections containing each bacterial species identified from the southern pine beetle.

Species	Female			Male		
	Foregut(10)[1]	Midgut(8)	Hindgut(7)	Foregut(6)	Midgut(9)	Hindgut(7
	--percent--			-percent		
Achromobacter						
erydice	10%	13%	0	17%	22%	14%
delmarvae	20	0	0	0	0	0
iophagus	0	0	14	0	11	0
guttatus	0	0	0	0	11	0
liqufaciens	0	0	0	0	11	14
superficialis	0	0	0	17	0	0
xerosis	20	0	29	17	44	14
strains A	0	13	14	0	11	14
B	0	0	0	0	11	29
C	0	13	0	0	22	0
D	20	13	14	33	11	14
E	0	0	0	0	11	0
F	10	0	0	0	11	0
G	10	0	0	33	0	14
H	0	0	0	0	22	14
Alcaligenes						
faecalis	20	25	43	33	22	29
metacaligenes	0	0	0	0	0	14
recti	10	13	14	0	0	29
viscolactis[2]	0	0	14	0	11	0
strains A	0	13	0	0	0	14
B	10	0	0	0	0	0
C	0	0	14	0	11	0
D	0	0	0	0	0	14
Brevibacterium						
acetylicum	10	13	14	0	22	43
incertum	0	0	14	0	11	0
lipolyticum[2]	10	0	0	0	44	43
quale	0	0	14	0	0	29
viterumen[2]	0	13	0	0	0	0
strains A	10	0	0	0	11	0
B	10	0	0	0	11	0
Pseudomonas						
strains A	0	0	0	0	0	29
B	20	25	14	0	33	14
C	0	0	0	0	11	0
D	0	0	0	17	11	0
E	10	0	0	0	0	0

(cont'd)

Table 2.--Percent of gut sections containing each bacterial species identified from the southern pine beetle (cont'd)

Species	Female			Male		
	Foregut(10)[1]	Midgut(8)	Hindgut(7)	Foregut(6)	Midgut(9)	Hindgut(7
	--percent--			--percent--		
Bacillus cereus	30	13	0	17	11	14
Bacillus megaterium	0	13	14	17	0	0
Sarcina lutea	0	25	14	0	0	0
Sarcina flava	0	0	0	33	0	0
Enterobacter cloaca	0	0	14	0	0	0
Enterobacter strains A	0	0	0	17	0	0
Enterobacter strains B	0	0	14	17	0	14
Flavobacterium strains A	0	0	14	0	0	0
Flavobacterium strains B	0	25	0	0	0	0
Flavobacterium strains C	10	0	0	0	0	0
Kurthia bessonii	0	0	0	0	0	14
Mycobacterium rhodochorous[2]	0	0	0	17	0	0
Staphylococcus unknown sp.	0	13	0	0	0	0

1/ Number of gut samples

2/ Tentative identification

Nineteen species or strains were recorded from only one location in the male or female gut system. All but one of these (Sarcina flava) were in less than 25% of the respective guts, suggesting no high degree of specificity for the beetle.

DISCUSSION

This study recorded 11 genera and 23 identified species in contrast to the 6 genera and 6 identified species found by Moore (1972) in the alimentary tract of healthy larvae and adults. surveyed in North Carolina. He reported that Aerobacter aerogenes, Alcaligenes faecalis, and Serratia spp. were the most frequently isolated bacteria. Three of Moore's 6 species, Alcaligenes faecalis, Bacillus cereus, and B. megaterium were isolated from Louisiana beetles in the present study. In addition both the NC and LA beetles contained Pseudomonas; however, Xanthomonas and Serratia were found only by Moore in NC. Enterobacter, formerly called Aerobacter, was found in Louisiana beetles but was recorded as Aerobacter in the North Carolina beetles.

Oberle (1966) recorded 5 genera and 9 species of bacteria from adult and larval galleries of the southern pine beetle in Louisiana. Three of these genera, Achromobacter, Flavobacterium, Pseudomonas,and two species, A. erydice and A. liquefaciens, were found in guts during the present study. A. erydice, found in all sections but the female hindgut, was most frequently isolated from galleries by Oberle. Serratia and Aerobacter were also found in galleries.

This study was not designed to determine the pathogenicity of the isolated organisms, but it should be noted that two species, Alcaligenes faecalis and Bacillus cereus, were recorded to be pathogenic to southern pine beetle larvae in laboratory tests (Moore 1972). In addition, two species of Pseudomonas were also pathogenic.

Many of the bacteria found in the southern pine beetle gut were previously isolated from 30 species of plant feeding insects by Steinhaus (1940). He reported 2 strains of Achromobacter, 3 of Pseudomonas, 3 of Alcaligenes, 8 of Flavabacterium, 9 of Bacterium (= Brevibacterium), and 12 of Bacillus and others.

The southern pine beetle guts sampled in this study thus contained a variety of genera and species found within various insects on various plant hosts. It is too early to determine what role these bacteria play in the ecology of the complex association of microbes in the gallery or in the development of the insects themselves. A future study, "Identification of bacterial flora in galleries of the southern pine beetle," FS-SO-2203-1.29, will shed light on those bacteria which may influence the host phloem substrate before and during larval feeding. A comparison of all bacteria associated with beetle development should also begin to provide an indication of the function of the various organisms.

LITERATURE CITED

Moore, G. E., 1972. Microflora from the alimentary tract of healthy southern pine beetles, Dendroctonus frontalis (Scolytidae), and their possible relationship to pathenogenicity. J. Invert. Path. 19: 72-75.

_____ 1972. Pathogenicity of ten strains of bacteria to larvae of the southern pine beetle. J. Invert. Path. 20: 41-45.

Oberle, A. 1966. The microflora isolated from galleries of southern pine bark beetles, Dendroctonus frontalis. MS thesis, Northwestern State College, Natchitoches, LA.

Steinhaus, E. A. 1941. A study of bacteria associated with thirty species of insects. J. Bacteriol. 42: 757-790.

APPENDICIES

Barnes SW a.s. '74

Appendix I: Species of bacteria isolated from the guts of female and male southern pine beetles

Achromobacter erydice
delmarvae
iophagus
guttatus
liquefaciens
superficials
xerosis
A. strains A-H

Alcaligenes faecalis
metacaligenes
recti
viscolatis
A. strains A-D

Brevibacterium acetylicum
incertum
lipolyticum
quale
*vitarumen**
B. strains A and B

Bacillus cereus
megaterium

Enterobacter (=*Aerobacter*) *cloacae*

E. strains A and B

Flavobacterium strains A-C

Kurthia bessonii

*Mycobacterium rhodochrous**

Pseudomonas strains A-E

Sarcina lutea
flava

Staphylococcus strain A

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*Tentative identification

Appendix II: Species of bacteria isolated from three gut sections of female adult beetles.

Beetle No.	Foregut	Midgut	Hindgut
1	Achromobacter "D" Pseudomonas "B"	Achromobacter "D"	Achromobacter xerosis "A" "D" Alcaligenes faecalis "C" Bacillus megaterium Brevibacterium acetylicum incertum quale Enterobacter cloaca "B" Flavobacterium "A" Unidentified bacteria
2	Pseudomonas "B"	Achromobacter erydice sp. "A" Bacillus cereus Brevibacterium acetylicum Flavobacterium "B" Sarcina lutea Staphylococcus "A"	Pseudomonas "B"

(cont'd.)

Appendix II: Species of bacteria isolated from three gut sections of female adult beetles. (cont'd.)

Beetle No.	Foregut	Midgut	Hindgut
3	Alcaligenes faecalis	Alcaligenes faecalis Pseudomonas "B"	Alcaligenes faecalis
4	Alcaligenes faecalis recti Brevibacterium acetylicum	Achromobacter "C" Alcaligenes "A" Bacillus megaterium Pseudomonas "B"	Alcaligenes recti
5	Pseudomonas "E"	Alcaligenes recti	Achromobacter xerosis Alcaligenes faecalis
6	Bacillus cereus Brevebacteria lipolyticum[a]	Alcaligenes faecalis	Alcaligenes viscolactis
7	Flavobacter "C"	Flavobacter "B" Sarcina lutea	Achromobacter iophagus Sarcina lutea

(cont'd.)

Appendix II: Species of bacteria isolated from three gut sections of female adult beetles. (cont'd.)

Beetle No.	Foregut	Midgut	Hindgut
8	Achromobacter xerosis "F" Alcaligenes "B" Bacillus cereus Brevibacterium "B"	—[b]	—
9	Achromobacter delmarvae erydice xerosis "D" "G" Bacillus cereus	—	—
10	Achromobacter delmarvae Brevibacterium "A"	—	—
11	—	Brevibacterium vitarumen[a]	—

a Tentative identification
b Not recovered for plating

Appendix III: Species of bacteria isolated from three gut sections of male adult beetles.

Beetle No.	Foregut	Midgut	Hindgut
1	Bacillus cereus Sarcina flava	Achromobacter guttatus iophagus xerosis "B" "D" "H" Brevibacterium acetylicum lioplyticum	Achromobacter erydice liquefaciens Bacillus cereus Brevibacterium quale
2	Bacillus megaterium	Achromobactera erydice liquefaceins "A" "C" Brevibacterium acetylicum lipolyticum "B" Pseudomonas "D"	Achromobacter "B" Brevibacterium acetylicum Pseudomonas "A"
3	Achromobacter superficialis Alcaligenes faecalis	Alcaligenes faecalis Pseudomonas "B"	Achromobacter sp. H Alcaligenes faecalis Brevibacterium lipolyticum* Kurthia bessonii

(cont'd)

Appendix III. Species of bacteria isolated from three gut sections of male adult beetles. (cont'd)

Beetle No.	Foregut	Midgut	Hindgut
4	Alcaligenes faecalis Sarcina flava	Achromobacter xerosis Brevibacterium "A" Pseudomonas "B"	Achromobacter "G" Alcaligenes faecalis "D" Pseudomonas "A"
5	Achromobacter sp. G Mycobacterium rhodochrous[a]	Pseudomonas sp. C	Pseudomonas "B"
6	——[b]	Achromobacter erylice xerosis "F" Alcaligenes "C" Bacillus cereus Brevibacterium incertum lipolyticum[a]	Achromobacter xerosis sp. A sp. D Alcaligenes metacaligenes recti sp. A Brevibacterium acetylicum lipolyticum quale
7	Achromobacter erydice xerosis "D" Enterobacter "A" "B" Pseudomonas "C"	Achromobacter xerosis "E" "H"	——

(cont'd.)

Appendix III: Species of bacteria isolated from three gut sections of male adult beetles. (cont'd)

Beetle No.	Foregut	Midgut	Hindgut
8	___	Alcaligenes viscolactis[a] faecalis Brevibacterium lipolyticum[a] Pseudomonas "B"	Achromobacter "B" Brevibacterium acetylicum lipolyticum[a] Enterobacter "B"
9	___	Achromobacter "C"	___

a Tentative identification
b Not recovered for plating